

# **O. APPENDIX 10.**

## **KPDES Permittee Report Form**

10/27/92  
DOW

Test Type: Acute \_\_\_\_\_ Screen \_\_\_\_\_  
Chronic \_\_\_\_\_ Definitive \_\_\_\_\_

**KENTUCKY TOXICITY TEST REPORT SHEET**

- 1) Facility/Discharger: \_\_\_\_\_ Report Date: \_\_\_\_\_
- 2) Address: \_\_\_\_\_
- 3) KPDES Permit #: \_\_\_\_\_ 4) Receiving Stream: \_\_\_\_\_
- 5) Facility Contact: \_\_\_\_\_ 6) Phone #: (     ) \_\_\_\_\_
- 7) Consultant/Testing Lab Name: \_\_\_\_\_
- 8) Lab Contact: \_\_\_\_\_ Phone #: (     ) \_\_\_\_\_
- 9) Outfall(s) Tested:  
Average daily flow  
on days sampled (MGD):
- 11) Test Species: #1 \_\_\_\_\_ #2 \_\_\_\_\_
- 12) Species Age: #1 \_\_\_\_\_ #2 \_\_\_\_\_
- 13) Organism Source: #1 \_\_\_\_\_ #2 \_\_\_\_\_
- 14) Acclimation Procedure: #1 \_\_\_\_\_  
#2 \_\_\_\_\_
- 15) Test Conditions: Static \_\_\_\_\_ Static-Renewal \_\_\_\_\_
- 16) Dilution Water Type (synthetic, receiving stream): \_\_\_\_\_
- 17) Aeration? (Before/During Test): \_\_\_\_\_
- 18) Dechlorination?: \_\_\_\_\_ Original Chlorine Level: \_\_\_\_\_

\_\_\_\_\_  
Signature of person filling out form

\_\_\_\_\_  
Date

\_\_\_\_\_  
Name (typed or printed)

\_\_\_\_\_  
Date

### Sampling Summary

Outfall	Type Grab/Composite	Volume Collected	Sample Begin MM/DD/Time	Collection End MM/DD/Time	Rain Event?

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### Dates/Times of Test Performance:

Species #1

Species #02

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(name)

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(name)

Grab #: \_\_\_\_\_

### Toxicity Test Results

Results of a \_\_\_\_\_ Toxicity Test  
(Genus) (Species) (Type/Duration)  
 Conducted \_\_\_\_\_ - \_\_\_\_\_ Using Effluent From Outfall \_\_\_\_\_  
(mm/dd/yy) (mm/dd/yy) (number)

Test Solution	Percent Surviving (time intervals used--day/hour)	# of Young		Dry Weight	
		Total	Mean	Total	Mean
Control	_____	_____	_____	_____	_____
_____ % Effluent	_____	_____	_____	_____	_____
_____ % Effluent	_____	_____	_____	_____	_____
_____ % Effluent	_____	_____	_____	_____	_____
_____ % Effluent	_____	_____	_____	_____	_____
_____ % Effluent	_____	_____	_____	_____	_____

LC <sub>50</sub> /IC <sub>25</sub> Value _____  95% Confidence Limits UL _____ LL _____  UL = Upper Limit LL = Lower Limit	Calculated TU Estimate* _____ (indicate acute/chronic)  Permit Limits _____ (indicate TU <sub>i</sub> /TU <sub>c</sub> )  If acute test, method used to determine LC <sub>50</sub> and Confidence Limit values:
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\*NOTE: TU<sub>i</sub> = 100/LC<sub>50</sub>; TU<sub>c</sub> = 100/IC<sub>25</sub>

Reference Toxicant Test Results					
Species	Date	Time	Duration	Toxicant	Results (LC <sub>50</sub> /IC <sub>25</sub> )
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____

## Additional Toxicity Test Information

- 1) Submit copies of all bench sheets and statistical calculations/printouts obtained during the test(s). Data must be presented in tabular form and must include all physical and/or chemical measurements recorded during the tests (e.g. temperature, conductivity, total residual chlorine, dissolved oxygen, etc.) .
- 2) Methods/Instrumentation used in chemical analysis:  
  
Dissolved oxygen:  
pH:  
Temperature:  
Conductivity:  
Alkalinity:  
Hardness:  
Total Residual Chlorine:  
EPA Acute/Chronic Manual Edition and Date:
- 3) Indicate below any other relevant information that may aid in the evaluation of this report. Include any deviations from EPA methodology that were necessary for these tests as well as any sample manipulations which were performed, such as aeration, dechlorination with sodium thiosulfate, etc. and the justification for such manipulations or deviations. Attach additional pages as needed.

## **P. APPENDIX 11.**

### **Split Sample Protocol**

## BIOMONITORING SPLIT-SAMPLE PROTOCOL

The purpose of split-sample biomonitoring is to verify test results. To that end, it is very important that sampling and testing protocols be done as consistently as possible so the test results from multiple laboratories may be compared. Given that a certain degree of inter- and intra-laboratory variability is inherent in all laboratory analyses, it is important to minimize those variables that can be controlled. To help deal with these variables, the Bioassay Section of the Division of Water recommends the following protocol to be strictly followed to minimize inter-laboratory variability. All steps must be carefully documented. Furthermore, the Division recommends that testing be performed by only one laboratory or by three -- not two laboratories. While we recognize the added financial burden of contracting three laboratories, this is in fact the minimum number of split-sample tests that can realistically be done to confirm or refute a particular set of data.

Split-sample protocol are as follows:

1. Sample collected and split simultaneously from same composite sample; chain-of-custody must be documented
2. Sample preserved at 4°C if used after 4 hours of collection (i.e. each grab or composite)
3. Sample used within 36 hours of collection and less than 4 hours difference between laboratory test initiation
4. Sample handling must be identical, especially regarding filtration, aeration, dechlorination, etc.
5. Identical dilution series must be used
6. Control water must be EPA moderately hard synthetic water or an approved substitute may be used by both laboratories (i.e. same control water for both laboratories)
7. Organism ages:
  - a. fathead minnow:
    - Acute: less than 2 days differences between laboratories
    - chronic: less than 24 hours old
  - b. *Ceriodaphnia dubia*:
    - acute: less than 24 hours old
    - chronic: less than 24 hours old and within an 8 hour time span of release (time span should be same for both laboratories, i.e. if lab #1 uses ceriodaphnids 4 - 12 hours old, lab #2 should do the same)
7. Temperature: 25°C +/- 1, and all other standard EPA test

requirements must be followed.

Valid tests (i.e. following permit test conditions) not conforming to the split-sample protocol shall be judged on their own merit and the maximum Toxicity Unit (TU) of these tests will be entered in the facility's permit compliance record. Best Professional Judgement (BPJ) shall be used to evaluate test results conforming to split-sample protocol where differences in test results occur.

02/08/93

## **Q. APPENDIX 12.**

### **Standard Additions For Alkalinity**

## Alkalinity Standard

0.5000  $\pm$  0.0004N  $\text{Na}_2\text{CO}_3$

Voluette Cat. No. 14278-10



HACH COMPANY, P.O. Box 907

Ames, Iowa U.S.A. 50010

Telephone: 515-232-2533 ■ TWX 910-520-1158

800-227-4224

This standard was prepared by weighing the required amount of anhydrous sodium carbonate, primary standard (purity 100.00  $\pm$  0.05%) to the nearest 0.1 mg. The solvent was double de-mineralized water. Class A glassware was used throughout. The maximum tolerances of the standard, due to reagent purity and errors in glassware calibration and weighing, are equal to  $\pm$  0.08% ( $\pm$  0.0004N or  $\pm$  0.02 mg/L alkalinity as  $\text{CaCO}_3$ ).

### Storage Precaution

Keep contents of ampules from freezing.

### Procedure

1. Determine the alkalinity of the test sample as described in your handbook or test kit procedure.
2. Snap the neck off a 0.500N Alkalinity Voluette® Ampule Standard. Using the TenSette™ Pipet, add 0.10 mL of standard to the sample titrated in the procedure. Swirl to mix.
3. Titrate the sample again to the same end points and record the alkalinity of the sample.

4. Add 0.20-mL and 0.30-mL increments of standard to the test sample, titrating to the same end points after each addition. In the Buret Method (50-mL sample) the alkalinity should increase 50 mg/L for each 0.1-mL increment of standard added. In the Digital Titrator Method (100-mL sample) each 0.1-mL addition should cause an increase in alkalinity of 25 mg/L.

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Instructions Cat. No. 14278-88

7/84

MADE IN U.S.A.

In analytical chemistry there is always one nagging question in the back of an analyst's mind: "Was the result of my last analysis correct?" A valuable technique for solving this problem is the method of standard additions, sometimes called "known additions" or "spiking."

To perform standard additions, an analysis is run on a sample and the result recorded. Then, a small amount of a standard solution (a standard addition) is added to a second portion of the sample. The test is repeated. The original sample analysis is assumed to be correct if the amount found in the second test is equal to all of the original value plus the added "spike."

For example, a 25-mL water sample was analyzed and found to contain 0.3 mg/L iron. A second 25-mL sample was then taken, and 0.1 mL of a 50-mg/L iron standard solution added to it. The second sample (with 0.1-mL spike) was then analyzed and found to contain 0.5 mg/L iron.

How does this prove the original answer of 0.3 mg/L iron was correct? The addition of 0.1 mL of a 50-mg/L standard is equivalent to:

1

$$0.1 \text{ mL} \times \frac{50 \text{ mg}}{\text{liter}} = \frac{5 \text{ mL (mg)}}{\text{liter}} \div 25 \text{ mL sample} =$$

$$\frac{5 \text{ mL (mg)}}{\text{liter}} \times \frac{1}{25 \text{ mL}} = \frac{0.2 \text{ mg}}{\text{liter}} = 0.2 \text{ mg/L}$$

The result of the first test subtracted from the result of the second test (0.5 mg/L minus 0.3 mg/L) equals 0.2 mg/L iron. Because 0.2 mg/L iron is equal to the amount of iron added in the standard addition, the analyst is reassured that the original result was correct.

If the second result had been more or less than 0.5 mg/L, the analyst would have reason to suspect that the first result probably was incorrect. Likely sources of error include deteriorated test reagent, interferences, improper colorimeter calibration, faulty analytical technique, contamination and dirty glassware. When trying to determine the cause of such errors, the standard addition technique is useful as a check, for correction of the faulty procedure will produce a corrected result of 0.5 mg/L iron.

2

$$\text{True} = \frac{\text{actual mg/L of standard addition}}{\text{observed mg/L of one standard addition}} \times \text{original mg/L found}$$

Using the above formula:

$$\text{True mg/L} = \frac{0.2 \text{ mg/L}}{0.1 \text{ mg/L} \times 0.3 \text{ mg/L}} = 0.6 \text{ mg/L}$$

#### Voluette® Ampule Standards For Standard Additions

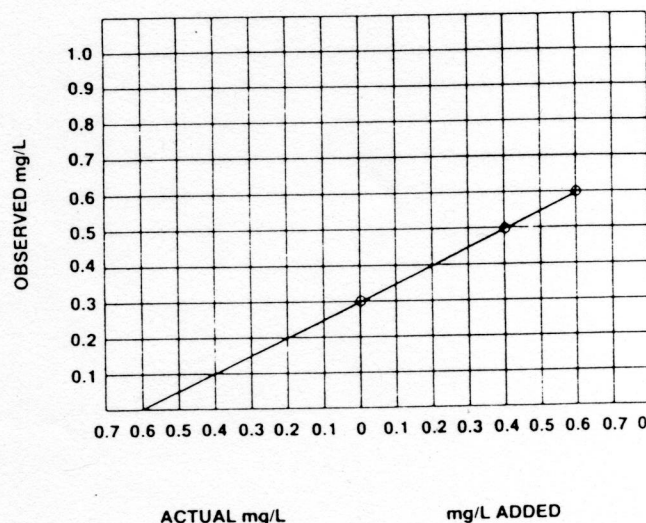
Test	Cat. No.	Concentration
Acidity	14330-10	0.500N H <sub>2</sub> SO <sub>4</sub>
Alkalinity	14278-10	0.500N Na <sub>2</sub> CO <sub>3</sub>
Aluminum	14792-10	50 mg/L Al
Ammonia	21284-10	150 mg/L NH <sub>3</sub> (for TKN)
Barium	14251-10	5000 mg/L Ba
BOD	14865-10	for dilution method, glucose plus glutamic acid at 300 mg/L each

If preferred, the true sample value also can be determined graphically. For example, a 25-mL water sample was analyzed and found to contain 0.3 mg/L iron. More samples were run; the results were 0.5 mg/L with a 0.2-mL (0.4-mg/L) iron standard solution spike, and 0.6 mg/L with a 0.3-mL (0.6-mg/L) spike. Something was causing poor recovery of the standard solution spikes. The first result (with the 0.2-mL spike) should have been 0.7 mg/L; the second result (with the 0.3-mL spike) should have been 0.9 mg/L. It is still possible to determine the true sample value by plotting the data on a graph. The analyst extends the line formed by the three data points and the vertical axis intercept backward through the horizontal axis to determine the actual iron concentration in the sample (0.6 mg/L iron). (See Figure.)

If the difference between each standard addition is approximately uniform, the true concentration can be calculated without the use of a graph. For instance, in the last example, each addition of standard iron solution should have produced an increase of 0.2 mg/L for every 0.1 mL added. Instead there was an increase of only 0.1 mg/L iron. The concentration may be found by placing the observed and known data in the following equation:

3

PLOT OF STANDARD ADDITION



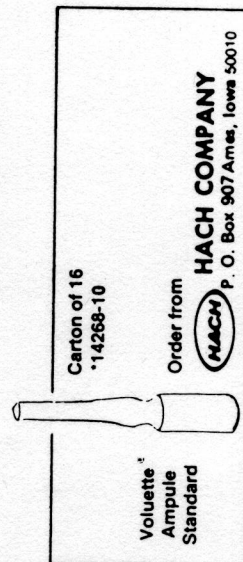
Test	Cat. No.	Concentration
BOD	14866-10	for manometric method, glucose plus glutamic acid at 3000 mg/L each
Boron	14249-10	250 mg/LB
Cadmium	14261-10	25 mg/L Cd
Calcium	14023-10	1000 mg/L Cd
Chloride	2187-10	10,000 mg/L CaCO <sub>3</sub>
	22403-10	1000 mg/L Ca
	183-10	1000 mg/L Cl <sup>-</sup>
Chlorine, Total, DPD	14250-10	12,500 mg/L Cl <sup>-</sup>
Chromate, Sodium	14268-10	50 mg/L Cl <sub>2</sub>
Chromium, Hexavalent Total and Trivalent	14255-10	25,000 mg/L Na <sub>2</sub> CrO <sub>4</sub>
	14256-10	12.5 mg/L Cr <sup>6+</sup>
	14257-10	12.5 mg/L Cr <sup>3+</sup>
Copper	21126-10	12.5 mg/L Cu <sup>2+</sup>
Expanded Low Range	14248-10	20 mg/L Cu <sup>2+</sup>
	14247-10	75 mg/L Cu <sup>2+</sup>
	2593-10	1000 mg/L Cu <sup>2+</sup>
Detergents	14271-10	60 mg/L LAS
Formaldehyde	22573-10	4000 mg/L as CH <sub>2</sub> O
Hardness, Total	2187-10	10,000 mg/L CaCO <sub>3</sub>
Iron, Total	14254-10	50 mg/L Fe
FerroVer®	14253-10	25 mg/L Fe
FerroZine®		

6

7

Test	Cat. No.	Concentration
Expanded Low Range	140-10	10 mg/L Fe
High Range	2271-10	1000 mg/L Fe
Lead	14262-10	50 mg/L Pb
	12796-10	1000 mg/L Pb
Manganese	21128-10	25 mg/L Mn
High Range	14258-10	250 mg/L Mn
Expanded Low Range	14264-10	100 mg/L Mn
Mercury	14263-10	125 mg/L Hg
	14195-10	1000 mg/L Hg
Nickel	14266-10	300 mg/L Ni
	14176-10	1000 mg/L Ni
Nitrogen	21091-10	160 mg/L NH <sub>3</sub> (N)
Ammonia	14791-10	50 mg/L NH <sub>3</sub> (N)
Ammonia Nitrate		
High Range	14260-10	500 mg/L NO <sub>3</sub> (N)
Low Range	14333-10	12 mg/L NO <sub>3</sub> (N)
Phosphate, Ortho		
High Range	14242-10	500 mg/L PO <sub>4</sub>
Low Range	171-10	50 mg/L PO <sub>4</sub>
Expanded Low Range	14243-10	15 mg/L PO <sub>4</sub>
Phosphorus	21092-10	25 mg/L P
Potassium		
High Range	21093-10	500 mg/L K
Low Range	14790-10	250 mg/L K

Test	Cat. No.	Concentration
Silica		
High Range	14244-10	250 mg/L SiO <sub>2</sub>
Low Range	1117-10	50 mg/L SiO <sub>2</sub>
Expanded Low Range	14245-10	20 mg/L SiO <sub>2</sub>
Sulfate	14252-10	2500 mg/L SO <sub>4</sub>
Sulfite	21854-10	5000 mg/L SO <sub>3</sub>
Sulfur	21684-10	625 mg/L S
Strontium	22408-10	1000 mg/L Sr
Volatile Acids	14270-10	62,500 mg/L acetic acid
Zinc	14246-10	25 mg/L Zn



\*Approximate value: Concentration varies with each lot.  
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Instructions Cat. No. 14343-00

8/85

8